REMARKS

I. Status of the Claims

Claims 18-27 are pending and under examination.

II. Formalities

The Examiner has accepted the new declaration filed February 2008. Additionally, the Examiner has acknowledged that the application is entitled to the priority of Italian application MI2003A001909, filed October 3, 2003.

III. Rejections Under 35 U.S.C. §102/103

Claims 18-27 have been newly rejected as allegedly being obvious over Schauer *et al.* (EP0752474, published Aug. 1, 1997, the '474 publication) in view of Fontana *et al.* (1999, *Animal Cell Technology*, p. 245-249).

The Examiner cites Fontana for describing homologous recombination of CHO cells using a plasmid comprising DNA sequences coding for biologically active domains of CNAH.

The Examiner cites Schauer for teaching production of CMP-N-acetylneuraminic acid hydroxylase-deficient cells (abstract), including CHO cells (citing page 6, line 29). The Examiner concludes that it is his belief that the teachings of Schauer encompass the region of nucleotides 787-1598 of the cDNA of CMAH as described in the present invention.

This new rejection is respectfully traversed. As an initial matter, the Examiner mischaracterizes Schauer. Schauer does not describe the hamster CMAH gene, protein, or CHO cells having the claimed deletion. As described on page 9 of the '474 publication, Schauer used mRNA from pig submandibular glands to identify a clone coding for only about 80% of the CMP-Neu5Ac hydroxylase, *i.e.*, the deduced amino acid sequence SEQ ID

NO:2, which is 422 amino acids in length (full-length hamster CMAH protein is 563 amino acids). The corresponding coding sequence described by Schauer is SEQ ID NO:1, 1265 nucleotide bases in length (full length hamster CMAH sequence is 1598 bases). Thus, Schauer describes only a portion of the pig CMP-Neu5Ac hydroxylase. Schauer cannot provide any teachings relating to the specifically claimed constructs, because the claimed methods call for deleting a portion of the CMAH gene including and between bases 787-1598, a region not described or contemplated by Schauer's pig CMAH fragment.

Furthermore, *Schauer does not describe deleting any precise region of the CMAH gene*, but instead only references generic replacement or insertion vectors in Figure 1A-B. Figure 1A depicts a double homologous recombination event where presumably the entire target gene is deleted and replaced with a selectable marker. Figure 1B depicts a single recombination insertion upstream of the target gene. Thus, Schauer is silent with regard to the CHO cells as called for in the present claims.

As pointed out in the response from January 29, 2008, Fontana describes only certain specific plasmid constructs for making CMAH knockouts where the deleted portion of the hamster CMAH gene is predicted to span the functional domains for the Rieske center and for the first mononuclear iron binding site (i.e., the deleted regions would span portions of exon 2 and exon 5, See Fig. 1-2). Fontana does not describe or suggest knocking out exons other than exons 2 and 5. Fontana only identified a portion of the hamster CMAH coding regions as spanning putative exons 2-9 as aligned with the known mouse, human, and pig CMAH sequences as shown in Fontana Figs. 1-2. Fontana only describes a partial cDNA coding sequence showing the deduced amino acid sequence containing residues 1-366 (See Fig. 2). Thus, Fontana provides no teachings with respect to a longer hamster CMAH cDNA sequence including exons 10-15, and no teachings with respect to a hamster CMAH protein of 564 residues.

Fontana also fails to teach knocking out exons 8 and 9. Instead, Fontana comments only on a naturally occurring deletion mutant in a murine CNAH (citing Koyama *et al.* 1996,

Glycoconj. J. (13):353-358) as corresponding to exon 8 in the hamster sequence. Thus, Fontana does not teach or suggest CHO cells with knock-outs of portions of exons 8 and 9.

In contrast to Fontana and Schauer, the pending claims require knocking out specific portions of the gene between exons 8 and 15, *i.e.*, the portion comprising the sequence including and disposed between bases 787 and 1598 of cDNA encoding for hamster CMAH (corresponding to the sequence of CMAH including and disposed between amino-acid 262 and amino-acid 532).

Fontana does not describe or suggest any CMAH knock-outs other than plasmid constructs for making putative CMAH knockouts where the deleted portion of the hamster CMAH gene would span portions of exon 2 and exon 5.

Furthermore, it is noted that gene targeting by homologous recombination in mammalian somatic cells is difficult and unpredictable. The present invention provides plasmids containing specific constructs that provide efficient removal of unwanted CMAH enzymatic activity in a mammalian cell strain (CHO cells) by gene targeting. The specification also provides techniques for deletion of both alleles by repeated transfection of the same homologous recombination plasmid and subsequent removal of selection marker cassettes needed for clone selection.

In contrast, Fontana merely describes a theoretical approach for gene targeting based on one plasmid for gene homologous recombination which contains a portion of the hamster CMAH coding sequence (a different portion from the portions claimed in the present application, as described above), with no precisely defined deletion boundaries and without any experimental description relating to obtaining the recombinant CHO cells, and without any confirmation of results. The approach described by Schauer is similarly theoretical and relates to incomplete pig sequences.

For a claim to be obvious under U.S. patent law, the Examiner must explain why the difference(s) between the prior art and the claimed invention would have been obvious to one of ordinary skill in the art. Additionally, the Patent Office must articulate the reason(s) why a skilled artisan "would have recognized" that the results of the prior art "were predictable" (*See* Examination Guidelines, Department of Commerce, *Federal* Register, 72(195):57529 (October 10, 2007)).

The new rejection under 35 U.S.C. §103 fails because nothing in Schauer cures the deficiency of Fontana, because neither reference, alone or in combination, discloses or suggests a CHO cell system utilizing a specific fragment of the CMAH gene (*i.e.*, the portion between bases 787 and 1598 of the cDNA of CMAH) to remove unwanted CMAH enzymatic activity by gene targeting.

In contrast to the claimed invention, Schauer only reports (See pg. 6, line 19) that "...for homologous recombination it is preferred to use a targeting vector containing as much hydroxylase homology as possible..." referring to Figure 1, which is simply a schematic representation of a generic homologous recombination process. Schauer is even more generic in its description of CMAH gene targeting than the deficient disclosure by Fontana.

As noted previously, gene targeting by homologous recombination technology in mammalian somatic cells is a difficult and time-consuming process, in part because the desired recombination is a rare event. (*See* for example, references 4, 10, 11 and 13 cited in the pending application).

Thus, assembling the claimed elements in the manner discovered by the inventors to arrive at the claimed CHO cells was not a mere combination that yielded predictable results. Reconsideration of the claims and withdrawal of the rejections is requested.

CONCLUSION

In view of the above amendments and remarks, it is respectfully requested that the application be reconsidered and that all pending claims be allowed and the case passed to issue. Applicants reserve the right to pursue the canceled and/or non-elected subject matter in one or more continuation or divisional applications.

If there are any other issues remaining, which the Examiner believes could be resolved through either a Supplemental Response or an Examiner's Amendment, the Examiner is respectfully requested to contact the undersigned at the telephone number indicated below.

Dated: July 23, 2008

Respectfully submitted,

By 

Amy G. Klamm, Ph.D.
Registration No.: 48,155
DARBY & DARBY P.C.
P.O. Box 770
Church Street Station
New York, New York 10008-0770
(212) 527-7700
(212) 527-7701 (Fax)
Attorneys/Agents For Applicant